

Claims

1. A method for producing fibrinogen comprising:  
providing a first DNA segment encoding a secretion signal operably linked to a fibrinogen A $\alpha$  chain, a second DNA segment encoding a secretion signal operably linked to a fibrinogen B $\beta$  chain, and a third DNA segment encoding a secretion signal operably linked to a fibrinogen  $\gamma$  chain, wherein each of said first, second and third segments is operably linked to additional DNA segments required for its expression in the mammary gland of a host female mammal;

introducing said DNA segments into a fertilized egg of a non-human mammalian species;

inserting said egg into an oviduct or uterus of a female of said species to obtain offspring carrying said DNA constructs;

breeding said offspring to produce female progeny that express said first, second and third DNA segments and produce milk containing biocompetent fibrinogen encoded by said segments;

collecting milk from said female progeny;

and recovering the fibrinogen from the milk.

2. A method according to claim 1 wherein said species is selected from the group consisting of sheep, pigs, goats and cattle.

3. A method according to claim 1 wherein each of said first, second and third DNA segments comprises an intron.

4. A method according to claim 1 wherein the molar ratio of said first, second and third DNA segments is within the range of 0.5-1:0.5-1:0.5-1.

5. A method according to claim 1 wherein each of said first, second and third DNA segments is operably linked

to a transcription promoter selected from the group consisting of casein,  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin and whey acidic protein gene promoters.

6. A method according to claim 1 wherein said first, second and third DNA segments are expressed under the control of a  $\beta$ -lactoglobulin promoter.

7. A method according to claim 1 wherein said introducing step comprises injecting said first, second and third DNA segments into a pronucleus of said fertilized egg.

8. A method according to claim 1 wherein said fibrinogen is human fibrinogen.

9. A method according to claim 1 wherein said second DNA segment comprises a sequence of nucleotides as shown in SEQ ID NO: 3 from nucleotide 470 to nucleotide 8100.

10. A method according to claim 1 wherein said second DNA segment comprises a sequence of nucleotides as shown in SEQ ID NO: 3 from nucleotide 512 to nucleotide 8100.

*Revised!*

11. A method of producing fibrinogen comprising:  
incorporating a first DNA segment encoding a secretion signal operably linked to an A $\alpha$  chain of fibrinogen into a  $\beta$ -lactoglobulin gene to produce a first gene fusion;  
incorporating a second DNA segment encoding a secretion signal operably linked to a B $\beta$  chain of fibrinogen into a  $\beta$ -lactoglobulin gene to produce a second gene fusion;  
incorporating a third DNA segment encoding a secretion signal operably linked to a  $\gamma$  chain of fibrinogen into a  $\beta$ -lactoglobulin gene to produce a third gene fusion;  
introducing said first, second and third gene fusions into the germ line of a non-human mammal so that said DNA segments are expressed in a mammary gland of said mammal

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or its female progeny and biocompetent fibrinogen is secreted into milk of said mammal or its female progeny ;

obtaining milk from said mammal or its female progeny; and

recovering said fibrinogen from said milk.

12. A method according to claim 11 wherein said mammal is a sheep, pig, goat or <sup>Cow</sup>bovine.

13. A method according to claim 11 wherein each of said first, second and third gene fusions comprises an intron.

14. A method according to claim 11 wherein the molar ratio of said first, second and third gene fusions introduced is within the range of 0.5-1:0.5-1:0.5-1.

15. A method according to claim 11 wherein said introducing step comprises injecting said first, second and third gene fusions into a pronucleus of a fertilized egg and inserting said egg into an oviduct of a pseudopregnant female to produce female offspring carrying said gene fusions in the germ line.

16. A method for producing fibrinogen comprising:  
providing a transgenic female non-human mammal carrying in its germline heterologous DNA segments encoding A $\alpha$ , B $\beta$  and  $\gamma$  chains of fibrinogen, wherein said segments are expressed in a mammary gland of said mammal and fibrinogen encoded by said segments is secreted into milk of said mammal;

collecting milk from said mammal; and  
recovering said fibrinogen from said milk.

17. A method according to claim 16 wherein said mammal is a sheep, pig, goat or <sup>Cow</sup>bovine.

*A* <sup>*nuclei*</sup> ~~nucleus~~ 18. A non-human mammalian embryo containing in its heterologous DNA segments encoding  $A\alpha$ ,  $B\beta$  and  $\gamma$  chains of fibrinogen.

19. A transgenic non-human female mammal that produces recoverable amounts of human fibrinogen in its milk.

20. A process for producing a transgenic offspring of a mammal comprising:

*Sub B2* providing a first DNA segment encoding a fibrinogen  $A\alpha$  chain, a second DNA segment encoding a fibrinogen  $B\beta$  chain, and a third DNA segment encoding a fibrinogen  $\gamma$  chain, wherein each of said first, second and third segments is operably linked to additional DNA segments required for its expression in a mammary gland of a host female mammal and secretion into milk of said host female mammal;

introducing said DNA segments into a fertilized egg of a mammal of a non-human species;

inserting said egg into an oviduct or uterus of a female of said non-human species to obtain an offspring carrying said first, second and third DNA segments.

*21* 21. A process according to claim *20.3* wherein said offspring is female.

*22* 22. A process according to claim *20.23* wherein said offspring is male.

*23* 23. A non-human mammal produced according to the process of claim *20.23*.

*24* 24. A non-human mammal according to claim *23.26* wherein said mammal is female.

*25* *non-human* 25. A female mammal according to claim *24.27* that produces milk containing biocompetent fibrinogen encoded by said DNA segments.

<sup>27</sup>  
~~26.~~ A non-human mammal according to claim ~~25~~ <sup>26</sup>  
 wherein said mammal is male.

<sup>27</sup> <sup>30</sup>  
~~27.~~ A non-human mammal carrying in its germline  
 DNA segments encoding <sup>human</sup> ~~heterologous~~ A $\alpha$ , B $\beta$  and  $\gamma$  chains of  
 fibrinogen, wherein female progeny of said mammal express  
 said DNA segments in a mammary gland to produce biocompetent  
 fibrinogen.

<sup>31</sup> <sup>30</sup>  
~~28.~~ A <sup>non-human</sup> ~~human~~ mammal according to claim ~~27~~ <sup>30</sup> wherein said  
 mammal is female.

<sup>32</sup> <sup>31</sup>  
~~29.~~ A <sup>non-human</sup> ~~human~~ mammal according to claim ~~27~~ <sup>31</sup> wherein said  
 mammal is male.

Add B<sup>3</sup>